ISSN: 2395 - 0471

International Journal of Clinical and Biomedical Research
**Review Article**

# RECURRENT PREGNANCY LOSS AND ASSOCIATION OF MTHFR, PAI-1 AND ACE GENE POLYMORPHISMS IN WOMEN

***SNEHLATA PANDEY[1], ARCHANA PANDEY[3], U.K. CHAUHAN[1], ARVIND TRIPATHI [1], JITENDRA TRIPATHI[1], SANJEEV DUBEY[2], RISHABH DEO SAKET[1]**

[1]Center of Biotechnology studies school of Environmental Biology APSU Rewa M.P. India.
[2]Department of Botany, Government Model Science College, Rewa, (M.P.) India.
[3]Department of Gynecology SGMH Rewa M.P. India.
*Corresponding author email: snehlatapandey23@gmail.com

**Received: 30th June 2015, Accepted: 26th July 2015.**

## ABSTRACT

Recurrent pregnancy loss (RPL) is a significant clinical problem that may occur before the 20th week of gestation. There is no general consensus on how many consecutive abortions are considered as RPL. The goal of this study is to investigate the correlation between recurrent miscarriage (RM) and common polymorphisms in angiotensin-converting enzyme *(ACE),* plasminogen activator inhibitor 1 *(PAI-1)* and Methylenetetrahydrofolate Reductase (MTHFR) genes among women experiencing RM. The literature existing in different population was searched and based on these finding we conclude that polymorphism in either one of these genes may increase chances of miscarriage.

**KEYWORDS:** Polymorphism, recurrent pregnancy, plasminogen activator inhibitor 1.

## INTRODUCTION

Miscarriage is the most common complication of pregnancy, which affects approximately 15% of all clinically recognized pregnancies in the general population. Recurrent miscarriage is defined as three or more consecutive pregnancy losses before the 20th week of gestation. There is a tendency to include those with two or more than two abortions in such category. Recurrent spontaneous miscarriages (RM) affect up to 5% of fertile couples and, many of them, the causes remain unexplained [1].

Pregnancy can lead to folate deficiency, therefore an increased intake of folic acid during pregnancy is recommended. Folates are involved in a series of reactions involving the transfer of a carbon atom, holding important roles in two processes: DNA synthesis and DNA methylation. The methylene tetrahydrofolate reductase (MTHFR) is the essential enzyme for the conversion of 5, 10-methylenetetrahydrofolate (5, 10-methylene-THF) to 5-methyltetrahydrofolate (5-methyl-THF). The mutations in the MTHFR gene are present in individuals with hyperhomocysteinemia, representing a risk factor for NTD; miscarriages in the first trimester of pregnancy, in literature, at the level of the MTHFR gene, two polymorphisms have been frequently described: C677T and A1298C. These polymorphisms have been associated with the termolabile form of the MTHFR gene that causes the accumulation of homocysteine in the circulation (hyperhomocysteinemia) and decrease in folic acid. MTHFR mutations is common worldwide with an estimated 10-25% prevalence among various ethnic backgrounds the risk of embryonic and fetal loss is increased if the MTHFR gene mutation is combined with additional thrombophilic factors [2].

PAI-1 gene is a key regulating factor in the fibrinolysis cascade. Recently, associations between polymorphisms in the PAI-1 and

ACE genes and PAI-1 plasma levels have been established. Endothelial PAI-1 expression is modulated by a 4G/5G polymorphism in the PAI-1 promotor, 675 bp upstream from the site of start of transcription. The expression of PAI-1 is also infuenced by angiotensin II plasma levels. Angiotensin II is a very potent vasoconstrictor that is generated by the angiotensin I converting enzyme (ACE), which is play role in blood pressure regulation. ACE expression is associated with a deletion (D)/insertion (I) polymorphism in ACE gene's intron 16. Comparable to the PAI-1 4G allele, the ACE D allele leads to an increased PAI-1 expression, in the result of reduced fibrinolysis[3].

The 'physiological remodeling' of spiral arteries throughout pregnancy is mediated by the reninangiotensin system (RAS), which is one of the main factors regulating blood pressure, fluid and electrolyte balance[4]. Angiotensin converting enzyme (ACE) encoded by the ACE gene, is a key component of the Renin Angiotensin system which mediating the conversion of angiotensin I to angiotensin II. *In vitro* and *in vivo* studies indicate that ACE interferes with hemostasis by different mechanisms, including an influence on fibrinolysis, platelet aggregation and blood clotting[5]. Though the human ACE gene contains a number of variable polymorphic regions that can be of potential use in genetic analysis of populations,[6] the insertion/deletion (I/D) polymorphism in intron 16, has been extensively investigated. During pregnancy, marked changes in hemostasis take place and ACE D/D genotype has been proposed as a new thrombophilic factor influencing pregnancy negative events[7]. In addition to the well-known vasomotor functions, RAS is also involved in key events of the inflammatory process by increasing vascular permeability[8] and contributing to the recruitment of inflammatory cells[9]. Regarding hemostasis, several reactions are modulated by the renin-angiotensin system, and evidence exists for an association between the ACE D/D genotype and increased risk of thrombotic events[10]. Moreover, ACE by bradykinin degradation reduces nitric oxide levels, therefore contributing to endothelial dysfunction.

The purpose of this study is to evaluate whether MTHFR, ACE and PAI-1 expression Levels affect the early pregnancies and to determine the prevalence of the MTHFR, ACE D and PAI-1 polymorphisms.

**Material and methods**

An electronic search of the databases was investigated covering the period from January 2000 through 2012 using the keywords miscarriage, spontaneous abortion, pregnancy loss and association of MTHFR, ACE and PAL genes. Further searches were then carried out using references cited in the identified papers. Searches were not circumscribed by date or by language if an English abstract was available. Studies were subsequently included in the review if the majority of women in a study sample (i.e., at least 51%) experienced an early miscarriage (i.e., before the 20th week of gestation). Qualitative studies, as noted, were included when helpful in formulating hypotheses.

**Miscarriage definition**

Miscarriage, or spontaneous abortion, is the natural termination of a pregnancy before the fetus is considered viable. Roughly 15%–20% of recognized pregnancies end in miscarriage with about three quarters occurring before the 12th week of gestation. Although clinicians generally consider pregnancies that spontaneously terminate prior to the 14th–16th weeks of gestation to be miscarriages. The time frames used in research studies have ranged most typically, from up to 20 weeks to 27 weeks of gestation. Miscarriage rates rise dramatically with age, from about 27% for women aged 25–29 to about 40% for women aged 40 to about 75% for women aged more than or equal to 45[11].

**MTHFR gene**

Methylenetetrahydrofolate reductase (*MTHFR*) is involved in the synthesis of 5- methylenetetrahydrofolate, which is a cofactor in the enzymatic formation of methionine from homocysteine. The gene encoding *MTHFR* has been mapped to chromosomal region 1p36.3. Mutations in MTHFR gene are associated with hyperhomocysteinemia and increased thrombotic tendency. A polymorphism of *MTHFR* (C677T)

leads to an alanine to valine amino acid substitution within the predicted catalytic domain of MTHFR [12].

Eighteen mutations have been reported so far in the MTHFR gene, the most common being C677T and A1298C mis-sense mutations which have been reported to induce milder form of MTHFR deficiency. C677T transition in exon 4 extensively studied in the West, makes the enzyme thermolabile with decrease in its enzymatic activity[13] due to dissociation of dimer into monomers and loss of FAD-binding capacity[14]. The other frequent MTHFR mutation in exon 7, i.e. A1298C transversion, is not associated with thermolability of the enzyme with no impact on plasma total homocysteine; it has not been elaborated. Compound heterozygosity for (677CT/1298AC) will have similar clinical impact as C677T homozygosity. An individual with a 677TT genotype is always reported to have 1298AA genotype and vice versa[15].

**ACE gene**

Angiotensin converting enzyme (ACE) encoded by the ACE gene, is a key component of the Renin Angiotensin system mediating the conversion of angiotensin I to angiotensin II. *In vitro* and *in vivo* studies indicate that ACE interferes with hemostasis through different mechanisms, including an influence on fibrinolysis, platelet aggregation and blood clotting. Though the human ACE gene contains a number of variables polymorphic regions that can be of potential use in genetic analysis of populations the insertion/ deletion (I/D) polymorphism in intron has been extensively investigated. During pregnancy, marked changes in hemostasis take place and ACE D/D genotype has been proposed as a new thrombophilic factor influencing pregnancy negative events. In addition to the well-known vasomotor functions, reninangiotensin system is also involved in key events of the inflammatory process by increasing vascular permeability and contributing to the recruitment of inflammatory cells. Regarding hemostasis, several reactions are modulated by the renin-angiotensin system, and evidence exists for an association between the ACE D/D genotype and increased risk of thrombotic events. Moreover, ACE by bradykinin degradation reduces nitric oxide levels, therefore contributing to endothelial dysfunction[12].

**PAL-1 gene**

PAI-1 is a key regulating component in the fibrinolysis cascade. Recently, associations between polymorphisms in the PAI-1 and ACE genes and PAI-1 plasma levels have been established.

Endothelial PAI-1 expression is modulated by a 4G/5G polymorphism in the PAI-1 promotor, 675 bp upstream from the start site of transcription. PAI-1 expression is also infuenced by angiotensin II plasma levels,that is a very potent vasoconstrictor, is generated by the angiotensin Iconverting enzyme (ACE), which is well known for its role in blood pressure regulation. ACE expression is associated with a deletion (D)/insertion (I) polymorphism in intron 16 of the ACE gene. Comparable to the PAI-1 4G allele, the ACE D allele leads to an increased PAI-1 expression, resulting in reduced fibrinolysis[16].

These two genetic variations have been related to various vascular diseases such as myocardial infarction and deep vein thrombosis[17-21] as well as to pregnancy-related disorders such as severe pre-eclampsia, pregnancy-induced hypertension, or serious pregnancy complications such as growth retardation and stillbirth[22-24].

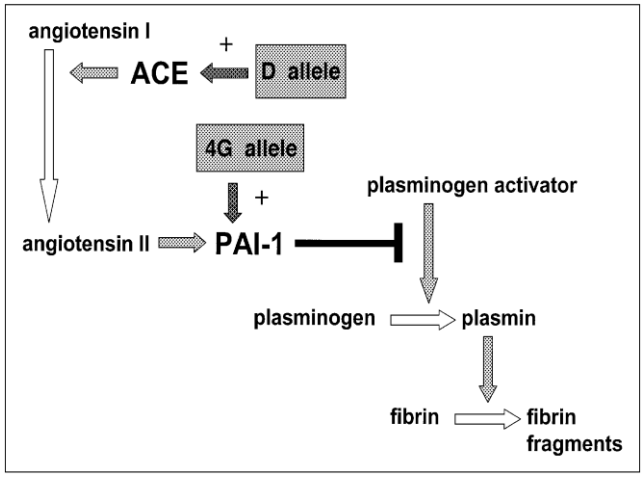


**Figure 1. A scheme of the angotensin/fibrinolysis pathway, illustrating the key role of angiotensin I-converting enzyme and plasminogen activator inhibitor-1. PAI-1 expression is increased by the 4G allele of the PAI-1 gene and by the D-allele of the ACE gene via angiotensin II.**

As both polymorphisms influence PAI-1 plasma levels and thereby plasmin and fibrin concentrations (Figure 1). This revealed a significant difference in the prevalence of the hypofibrinolytic combination of the PAI-1 4G/4G and ACE D/D genotypes, which was present in 13.6% of the RM patients, but only in 4.7% of the controls (P = 0.01). In contrast, individuals carrying the 4G/5G-D/I genotype (17.4% of the patients versus 28.3% of the controls; P = 0.02) appeared to have a lower risk of RM. The Cochran-Armitage trend test confirmed the relationship between a combination of the PAI 4G/4G and ACE D/D genotypes and RM (P = 0.02). Logistic regression analysis also demonstrated a significant influence of these two factors combined (P = 0.03), while the MTHFR C677T genotype were not significant variables. By stepwise logistic regression, the combination of the PAI-1 4G/4G and ACE D/D genotype was a significant positive explanatory variable for miscarriages (P = 0.01) and a significant independent risk factor for miscarriages with an odds ratio of 3.2[25].

## DISCUSSION

The relationships between three genetic polymorphisms and RPL were surveyed in this study. MTHFR is one of the most frequently studied thrombophilic genes controversially suspected to be associated with RPL.

The study by jeddi-tehrani et al. showed that the MTHFR 677C/T, and 1298A/C polymorphisms were found to be positively associated with RPL in Iranian women. Homozygosity but not heterozygosity for PAI-1)675 4G/5G polymorphism was significantly higher in patients with RPL than in the control group. The presence of these both mutations of MTHFR genes highly increased the risk of RPL. The data highlights the importance of thrombophilia screening in patients with RPL[26].

The results of the study by Daniela Neagos et al. showed that women with spontaneous abortion have a higher proportion of the 677TT genotype compared with the control group and the distribution of allelic and genotypic frequencies for polymorphism A1298C, does not present significant differences between the study group and control. The study also found that the frequency of the combined 677TT/1298 AA genotypes is higher in the study group compared with in women from Romania[27].

In another study homozygosity for the D allele of the ACE gene, which results in elevated PAI-1 concentrations and hypofibrinolysis, is associated with an elevated risk of RM and the combination of the D/D genotype with two 4G alleles of the PAI-1 promoter, which further increases PAI-1 plasma levels, is significantly more frequent in RM patients compared with controls demonstrated by[25].

V. Vettriselvi *et al*. study showed No statistically significant difference was observed in the distribution of genotypes between cases and controls for ACE and MTHFR polymorphisms in south Indian women further, the combination of MTHFR and ACE genotypes failed to reveal an association[12].

The Prevalence of methylene tetrahydrofolate reductase (MTHFR) gene mutations in South Indian population was investigated from a total of 608 samples, 420 adults and 188 newborns. Detection of mutation was carried out focussing on the two most common mutations of the MTHFR gene (C677T and A1298C) using PCRbased RFLP method. T-allele frequency was almost similar between the newborns and adults (0.0904, 0.1012). However, a higher T-allele frequency was observed in females (0.1538 and 0.12 in adults and newborns respectively) than males (0.0556 and 0.05 in adults and newborns respectively) by A. Radha Rama Devi et al.

Biochemical correlation of fasting plasma total homocysteine to MTHFR genotype revealed mild to moderate hyperhomocysteinemia in mutants. Plasma total homocysteine in males was found to be higher than in females in both normal and mutant individuals. TT homozygous women had higher plasma homocysteine. The high T-allele frequency, elevated plasma homocysteine and low folate intake in women could well be a risk factor for birth defects. The gender bias observed in this autosomal recessive trait was an interesting finding and is discussed[28].

The findings of Ajit K Saxena et al. study reveal that the highest (26.7%) incidence was observed in heterozygote (CT) cases when compared with controls (24.0%). The individual alleles

(T) frequency (0.13%) was also calculated by Hardy Weinberg equilibrium showing lack of significant differences ($p<0.05$). Biochemical analysis showed slight variation between homocysteine (17.02±14.64 μmol/l) and folates (16.76±8.48 ng/ml). Cytogenetic study showed chromosomal association between D and G – groups, while karyotype of one case is mosaic and was also observed. However, the calculated O.R. (1.15) suggests that "risk factor" increased to confirm that genotypic variants of MTHFR C677T gene polymorphism are responsible for fetal viability[29].

The study done by Hui chen et al.2014 found a significant association between the *PAI-1*-675G/A polymorphism and the risk of RPL under the recessive model (OR = 1.70, 95% CI = 1.21–2.38). However, no significant association between the *PAI-1*-844G/A polymorphism and RPL was noted. *PAI-1*-675G/A (4G/5G) polymorphisms play a potential role in RPL[30].

The result of a study by Unfried et al.2002 shows *MTHFR* allele frequencies in women with recurrent miscarriage and controls were 34.6% and 21.6%, respectively, for the T allele (mutant) and 65.4% and 78.4%, respectively, for the C allele (wild type) (*P* = .007, odds ratio 1.9, 95% confidence interval 1.2, 3.1). The *MTHFR* genotype frequencies in women with idiopathic recurrent miscarriage and controls were: 17.3% (T/T), 34.6% (C/T), 48.1% (C/C) and 5.4% (T/T), 32.4% (C/T), 62.2% (C/C), respectively (*P* = .03, odds ratio 3.7, 95% confidence interval 1.2, 11.8 [T/T versus C/T and C/C]). Serum concentrations of homocysteine were significantly higher in carriers of a *MTHFR* mutant allele compared with women with no mutant[31].

In the *ACE* polymorphism D/D genotype was less prevalent in RM patients (49%) than in controls (54%) but the difference was not significant (p = 0.479). The patients appeared to have a 1.41 times higher I/D genotype prevalence than the control subjects with a risk factor of 1.14, although there was no significant difference (p = 0.244). There was no significant difference in frequency between the genotype of the patient and control groups (p = 0.469). The results show no significant difference between the groups. Unexpectedly, 4G/5G appears to be more frequent in the controls[32].

In addition, many more studies have reported the association of MTHFR, ACE and PAL 1 polymorphism and increased risk for RPL. On the other hand, several groups have documented a lack of association between this polymorphism and RPL.

## REFERENCES


1. Coulam C.B., Clark D.A., Beer A.E. Current clinical options for diagnosis and treatment of recurrent spontaneous abortion. Clinical Guidelines Recommendation Committee for Diagnosis and Treatment of Recurrent Spontaneous Abortion. Am. J. Reprod. Immunol. 1997, 38, 57-74.
2. Rozano GA, Papadakis E, Brenner B. Combined Thrombophilia and Obstetric Complications. The Open Atherosclerosis & Thrombosis Journal. 2009; 2:38–41.
3. Kim D.K., Kim J.W., Kim S. Polymorphism of angiotensin converting enzyme gene is associated with circulating levels of plasminogen activator inhibitor-1. Arterioscler. Thromb. Vasc. Biol. 1997, 17, 3242-3247.
4. Griendling KK, Murphy TJ, Alexander RW. Molecular biology of the renin Angiotensin system. *Circulation* 1993; 87: 1816–1828.
5. Vaughan DE. Fibrinolytic balance, the renin-angiotensin system and atherosclerotic disease. *Eur Heart J* 1998; 19: 9–12.
6. Rigat B, Hubert C, Alhenc-Gelas F, Cambien F, Corvol P, Soubrier F et al. An insertion-deletion polymorphism in the angiotensin I-converting enzyme gene accounting for half the variance of serum enzyme levels. *J Clin Invest* 1990; 86: 1343–1346.
7. Mello G, Parretti E, Gensini F. Maternal-fetal flow, negative events, and preecalmpsia. Role of ACE I/D polymorphism. *Hypertension* 2003; 41: 932–937.
8. Pastore L, Tessitore A, Martinetti S. Angiotensin II *stimulates* intercellular adhesion molecole-1 (ICAM-1) expression by human vascular endothelial cells and increases solubile ICAM-1 release in vivo. *Circulation* 1999; 100: 1646–1652.
9. Suzuki Y, Ruiz-Ortega M, Lorenzo O, Ruperez M, Esteban V, Egido J. Inflammation and angiotensin II. *Int J Biochem Cell Biol* 2003; 35: 881–900.
10. Fatini C, Gensini F, Sticchi E. ACE DD genotype: an independent predisposition factor to venous thromboembolism. *Eur J Clin Invest* 2003; 33: 642–647.

11. Norman Brier. Grief Following Miscarriage: A Comprehensive Review of the Literature. Journal of women's health; 2008; 451-464.
12. Vettriselvi, Krishnaswami Vijayalakshmi, Solomon F. D. Paul, Perumal Venkatachalam. ACE and MTHFR gene polymorphisms in unexplained recurrent pregnancy loss. *J. Obstet. Gynaecol. Res. Vol. 34, No. 3: 301–306, June 2008.*
13. Frosst, P. A candidate genetic risk factor for vascular disease: A common mutation in methylenetetrahydrofolate reductase. *Nature Genet*. 1995, 10, 111–113.
14. Yamanda, K. Effects of common polymorphisms on the properties of recombinant human methylenetetrahydrofolate reductase. *Proc. Natl. Acad. Sci. USA* 2001, 98, 14853–14858.
15. Vanderput, N. M. J. A second common mutation in the methylenetetrahydrofolate reductase gene: An additional risk factor for neural tube defects. *Am. J. Hum. Genet*. 1998, 62, 1044–1051.
16. Kim D.K., Kim J.W., Kim S. Polymorphism of angiotensin converting enzyme gene is associated with circulating levels of plasminogen activator inhibitor-1. Arterioscler. Thromb. Vasc. Biol. 1997, 17, 3242-3247.
17. Sartori M.T., Wiman B., Vettore S. 4G/5G polymorphism of PAI-1 gene promoter and fibrinolytic capacity in patients with deep vein thrombosis. Thromb. Haemost. 1998, 80, 956-960.
18. Iacoviello L., Burzotta F., Di Castelnuovo A. The 4G/5G polymorphism of PAI-1 promoter gene and the risk of myocardial infarction: a meta-analysis. Thromb. Haemost. 1998, 80, 1029-1030.
19. Seino Y., Ikeda U., Maeda Y. Angiotensin-converting enzyme gene polymorphism and plasminogen activator inhibitor 1 levels in subjects with cerebral infarction. J. Thromb. Thrombolysis, 1998, 5, 263-267.
20. Gardemann A., Lohre J., Katz N. The 4G4G genotype of the plasminogen activator inhibitor 4G/5G gene polymorphism is associated with coronary atherosclerosis in patients at high risk for this disease. Thromb. Haemost. 1999, 82, 1121-1126.
21. Lane D.A., Grant P.J. Role of hemostatic gene polymorphisms in venous and arterial thrombotic disease. Blood, 2000, 95, 1517-1532.
22. Zhu M., Xia Y., Cheng W. Study on a deletion polymorphism of the angiotensin converting enzyme gene in pregnancy induced hypertension. Chung. Hua. Fu. Chan. Ko. Tsa. Chih. 1998, 33, 83-85.
23. Glueck C.J., Phillips H., Cameron D. (2000) the 4G/4G polymorphism of the hypofibrinolytic plasminogen activator inhibitor type 1 gene: an independent risk factor for serious pregnancy complications. Metabolism, 49, 845-852.
24. Yamada N., Arinami T., Yamakawa-Kobayashi, K. The 4G/5G polymorphism of the plasminogen activator inhibitor-1 gene is associated with severe preeclampsia. J. Hum. Genet. 2000, 45, 138-141.
25. T.Buchholz. ACE and PAI-1 polymorphisms associated with recurrent miscarriage.Human Reproduction. 2003, Vol.18, No.11 pp. 2473-2477.
26. Jeddi-tehrani . Analysis of Plasminogen Activator Inhibitor-1, Integrin Beta3, Beta Fibrinogen, and Methylenetetrahydrofolate Reductase Polymorphisms in Iranian Women with Recurrent Pregnancy Loss, 2010; 1-8.
27. Daniela Neagos. Investigation of the relationship between the risk of spontaneous abortion and C677T and A1298C polymorphisms of the methylenetetrahydrofolate reductase gene.2012, 335-343.
28. A.Radha Rama Devi. Prevalence of methylene tetrahydrofolate reductase polymorphism in South Indian population.2003, 440-443.
29. Ajit K Saxena, S Pandey, LK Pandey. Evaluation of methylenetetrahydrofolate reductase C677T gene polymorphism associated risk factor in the patients of recurrent pregnancy loss. 2012, 25-28.
30. Hui chen. Association between Plasminogen Activator Inhibitor-1 Gene Polymorphisms and Recurrent Pregnancy Loss: A Systematic Review and Meta-Analysis. 2014.
31. Gertrud Unfried, Andrea Griesmacher, Wolfgang Weismuller, Fritz Nagele, Johannes C. Huber, Clemens B. Tempfer. The C677T Polymorphism of the Methylenetetrahydrofolate Reductase Gene and Idiopathic Recurrent Miscarriage".2002; 614-619.
32. Al Sallout /Sharif. Polymorphisms in *NOS3, ACE* and *PAI-1* Genes and Risk of Spontaneous Recurrent Miscarriage in the Gaza Strip. 2010; 100-104.